Biological Oxidation and Cancer

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Life is driven by energy, the main source of which is biological oxidation. The oxidant in this process is the molecular oxygen of the atmosphere, O_2, the molecules of which have two uncoupled electrons. If these electrons spin in the opposite directions, then the molecules are in the singlet state, $^1O_2$. If they spin in the same direction, then they are in the triplet state, $^3O_2$. As emphasized by Khan and Kasha [1,2], the properties of the two are very different. $^1O_2$ is a powerful oxidizing agent the existence of which is incompatible with life, while $^3O_2$ is but moderately reactive. The O_2 of the atmosphere is in the triplet state. It follows that when it has to be used for biological oxidation, it has to be activated to the singlet state. However, once activated to this state, its oxidative power has to be utilized immediately, or else it would damage the living system. It follows that the substance which activates it also has to utilize the created oxidation power immediately, transmitting it to a third substance which can store it. The substance which activates the O_2 is a transition-metal atom, while the substance to which this metal transmits the oxidizing power is a polyphenol which is oxidized to a polyquinone. This polyquinone is itself an oxidizing agent which, then, can act as electron acceptor in metabolism. In this reaction the explosive oxidizing power of the singlet is traded in for a lower but more steady oxidizing power of quinones. The complex of O_2, metal, and polyphenol forms one single unit in which the electrons have a high degree of mobility which allows their rapid redistribution. The metal atoms thus not only activate O_2, but also act as a bridge between O_2 and polyphenol on which the electrons can pass.

Plants and animals are but recent leaves of the age-old tree of life, and so one would not expect to find an essential difference between them in such a fundamental process as biological oxidation. Plants are much simpler. For this reason I always have worked with preference on plants. One of my earliest papers, written six decades ago, dealt with oxidation in potatoes [3]. Then it was known that the transition metal responsible for the activation of oxygen in this material was copper, and I could show that the central polyphenol of the oxidation system was an orthodihydroxyphenol, a catechol derivative. The orthodiquinone formed on its oxidation was easy to detect, this quinone being a strong oxidizing agent which oxidized benzidine on quaiac resin to highly colored oxides. It also formed dark colored complexes with proteins. This means that the potato and other plants that have a catechol as a central polyphenol, all assume a dark color when their structure is damaged and their oxidation system is activated.

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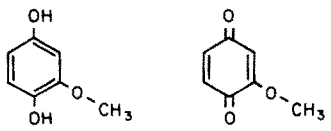
Another rather extensive group of plants does not show a dark coloration on damage. After having finished with the potato I tried to find out what the central polyphenol in these plants was. I failed, having no colors to lead me. The discovery of ascorbic acid was a by-product of this work. The lack of colors indicated that the polyquinone formed on oxidation of the polyphenol had a very low oxidation potential. Success on this line depended thus on finding a substance which could indicate a weak oxidation power. Such a substance is paraphenylene diamine (PD), which is oxidized by weak oxidizing agents to a blue imine. This makes the PD into a good reagent for the detection of a low oxidation potential. At the beginning of this century Battelli and Stern worked a great deal on this reaction, which they ascribed to the action of a specific enzyme, a "PD-oxidase" [4]. Their work received much attention which soon faded, PD not being a physiological substrate.

To demonstrate a low oxidation potential by PD in tissues, the tissue has to be mashed and thoroughly extracted with water to eliminate the electron donors present which could reduce the oxidized PD. The fact that thoroughly extracted tissue still gives a blue color with PD indicates that the oxidation is strongly bound to the insoluble cellular structure.

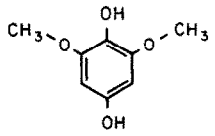
Having a reagent in hand for the detection of a low oxidation potential, the next problem was to find a material which was rich in the substance that gave the potential and colored PD. Such a material could make the isolation of the underlying substance a hopeful proposition. Wheat germ was found to be such a material. Wheat is a domesticated grass, and grass belongs to the group of plants which do not turn dark on damage and have no catechol for central polyphenol. Wheat is a main component of our daily bread. It is the fruit of this grass. It contains the germ in which the plant concentrates the substance essential for life. This germ can be obtained at a low price, in any quantity, at the grocery store.

More than three decades ago Cosgrove and his associates, interested in the baking of bread, made a chemical study of wheat germ and found in it a small quantity of methoxybenzoquinone (MB in Fig. 1) and a still smaller quantity of 2,6-dimethoxybenzoquinone (di-MB), which may have been the oxidation product of the corresponding hydroquinone [5,6]. On my request the Swedish pharmaceutical company Astra kindly synthetized for me methoxyhydroquinone (MH).* If $10^{-4}M$ of this MH (Fig. 1) was added to a 0.5% solution of PD at pH 7.4, the solution developed a purplish-blue color. $10^{-4}M$ of Cu^{+} or Mn^{2+} greatly increased the rate of this coloration. Fe^{2+} was found to have a very poor activity. MH behaved thus as a "PD-oxidase" and could be the central polyphenol of the oxidation system of animal tissues. The third oxygen introduced into the molecule by the methoxy group enabled the hydroquinone to bind metal and, through this metal, to bind O_2 and activate it. In themselves, transition

* I expected (and still expect) MH to have a virucidal activity and to prevent colds, and I wanted to test the substance for its ability to prevent or cure virus diseases. Astra kindly prepared the quantity of this substance needed, but I was unable to find the collaboration necessary for the testing.



MH MB



2:6 di-MH

Figure 1.

metals do not bind and activate oxygen. They are enabled to do so by being bound to the MH. When the O_2 is activated, this MH is oxidized to the corresponding quinone (MB in Fig. 1). There is, in all probability, also a change in the valency of the metal. It is very likely that in this form the MB–metal complex does not activate oxygen. This could explain how nature avoids the production of superfluous $^1O_2$, which is not needed as long as the MH is in the oxidized state.

If one methoxy group turned hydroquinone into a very active catalyst, MH, then it was reasonable to expect that two methoxy groups will do the same more strongly, and di-MH will be a still stronger catalyst than MH. Instead of increasing the activity, the second methoxy group abolished it. The second methoxy group was evidently not introduced into MH to increase its activity. It may have been introduced to serve as a handle by which the MH could be bound to the protein structure. By this binding the second methoxy group had to lose its inhibitory action.

As shown by the study of the electron spin resonance signals of MH by Gascoyne, the oxygen of the methoxy group takes part in the electronic changes accompanying the activation of oxygen. This seems important because it opens the possibility that through the bound methoxy group of di-MH the singlet oxygen desaturates the protein electronically, making its valence band conductant, establishing a direct connection between O_2 and the electronic state of protein.

Taken together, all this suggests that MH is actually the central polyphenol of the biological oxidation in the tissues that do not darken on damage, which includes the animal tissues. It fulfills this role by being oxidized by the metal complex of singlet oxygen to MB, which then acts as the central electron acceptor of metabolism.

Warburg has shown that in cancer the balance between oxidation and fermentation is shifted in favor of fermentation [7]. He thought that this change is the most important factor of malignant transformation and has to induce

malignancy by itself. He failed to clarify the chemical mechanism of these relations. Experiments conducted with McLaughlin suggest that cancer, and the failure of oxidation, might be due to the inability to synthetize MH. This failure, in its turn, may have been due to a disturbance in DNA. This assumption was supported by the fact that the only tissues that failed to oxidize PD in my experiments were the malignant tissues. While normal animal tissues gave a strong coloration with PD, the three cancer strains at my disposal (Ehrlich mammary carcinoma, sarcoma 180, and Morris hepatoma No. 3924 A) failed to do so.

If cancer is due to the lack of MH, then cancer should be inhibited or cured by administration of MH. What complicates the demonstration of such an activity is the fact that the cancer cells are covered by microvilli which may interfere with the penetration of the administered MH. Knock et al. [8] showed that large doses of ascorbate make the microvilli disappear and expose the surface of the cancer cell. My experiment showed that ascites tumor can be arrested in mice by the simultaneous administration of large doses of ascorbic acid and di-MH. Monomethoxyhydroquinone was found to be inactive, having no second methoxy group by which it could be bound to the protein. As scurvy is the expression of a lack of ascorbic acid, so most cancers may be the expression of a lack of di-MH.

MB oxidizes SH, and so the lack of MH may be responsible for the high SH content of cancer.

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